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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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VIKSNINS HARRIS & PADYS PLLP
P.O. BOX 111098
ST. PAUL, MN 55111-1098

EXAMINER	
MCGILLEM, LAURA L	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 08/10/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/694,520	BISHOP ET AL.	
	Examiner	Art Unit	
	Laura McGillem	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *9/29/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

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DETAILED ACTION

It is noted that this Office Action contains rejections of the same claims under 35 USC 112, 1st (written description) and 35 USC 103(a). While these rejections may seem contradictory, they are not, because each is based upon a different legal analysis, i.e., sufficiency of the disclosure of the instant application to support claims under 35 USC, 1st paragraph vs. sufficiency of a prior art disclosure to anticipate or render obvious an embodiment(s) of the claimed invention (See *In re Hafner*, 161 USPQ 783(CCPA 1969)).

Priority

This application receives priority of provisional application 60/422,674 filed 10/30/2002.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 30-31 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. Claims 30-31 are drawn to somatic cells comprising gene targeting vectors. The cells can be in a human and claims reading on *in vivo* human tissue are non-statutory because the claims read on part of a living human being *in situ*. Redrafting the claims to recite an " isolated somatic cell" would be remedial.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-31 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants claim vectors and methods for disruption of a gene of interest using a gene targeting construct comprising a positive negative selection (PNS) vector with a promoterless positive expression vector and a promoter operably linked to a negative selection marker. The claims read on a somatic cell gene targeting vector for use in the said method where the gene of interest is any gene in any somatic cell including such diverse cell types as somatic stem cells, neurons or hepatocytes. Applicants exemplify the use of this vector and method to disrupt Tumor necrosis factor receptor-associated factor (TRAF) expression in B cell lines and in fibroblasts.

The written description requirement for a genus may be satisfied by sufficient description of a representative number of species by actual reduction to practice or by disclosure of relevant identifying characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying

characteristics, sufficient to show that applicant was in possession of the claimed invention.

In the instant case, applicants provide no disclosure of any other gene which has been successfully targeted and disrupted in any cell other then the exemplified TRAF in B cell or fibroblasts. Given the very large number of diverse genes which could be targeted in an equally large number of cell types, the ability to disrupt the gene of interest would have to be determined empirically via gene expression studies. Applicants provide no correlation between the structure of the TRAF gene including length of the gene, position on the chromosome and strength of its corresponding promoter and the function of the TRAF gene comprising targeting vector. Additionally, the Applicants provide no disclosure as to the structure or sequences of any other gene in any other somatic cell type which the skilled artisan would expect to be disrupted using the claimed vector and methods. It is noted in a review by Wang and Zhou (Reprod Biol Endocrinol. 2003. Vol. 1(1): 103-111(pp.1-8) that not all genes are able to undergo homologous recombination using a selection method equally, due to length of the targeted gene, its location of the chromosome and proximity to a strong promoter (See page 3 of 8, right column, paragraph 2, in particular). In addition, Norgren (Reprod Biol Endocrinol. 2004. Vol.2:40-48 (pp1-8) teaches that not all gene targeting events result in functional disruption of the gene of interest (See page 5 of 8, left column, paragraph 2, in particular). In fact, Wang and Zhou state that some genes are impossible to target using promoterless targeting vectors due to the lack of active promoters for some genes in some cell types (see page 6 of 8, left column, conclusion

paragraph, for example). Given the above analysis, it must be concluded that the skilled artisan would not believe that the example of the TRAF gene which has been disrupted in two cell types would be a representative number of examples sufficient to describe the claimed genus of somatic cell gene targeting vectors.

Claims 13-31 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for *in vitro* methods of disrupting a TRAF gene, does not reasonably provide enablement for *in vivo* methods of disrupting any gene of interest or *in vitro* methods of disrupting any non-TRAF gene of interest in any cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Applicants claim methods for disrupting gene of interest in somatic cells using a gene targeting vector with positive and negative selection markers. The claims as written encompass broad uses of the methods for gene targeting including *in vivo* gene therapy in somatic cells or alteration of somatic cell genomes for further use nuclear transfer cloning methods.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the application coupled with information known in the art without undue experimentation *United States v. Telectronics*, Inc., 8 USPQ2d 1217 (Fed. Cir. 1988). Whether undue experimentation is required is not based upon a single factor, but rather is a conclusion reached by weighing many factors. These

factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter. 1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

1) Unpredictability of the art. Methods for gene targeting using positive negative selection (PNS) vectors *in vivo* or *ex vivo* in order to disrupt genes via homologous recombination in somatic cells is unpredictable. The unpredictability is manifested in the efficiency of homologous recombination of various genes. For post-filing date reviews, please see Wang and Zhou (cited above), Norgren (cited above) and Yanez and Porter (Gene therapy. 1998. Vol. 5:149-159). In a review published post-filing date of the instant application, Wang and Zhou describe several factors which influence homologous recombination in mouse embryonic stem cells and cite the need for increased efficiency in somatic stem cells. Wang and Zhou teach that variations to successful homologous recombination involve: 1) length of the sequences included in the targeting constructs 2) whether or not the targeted DNA is isogenic, meaning that the DNA comes from one or more organisms with the exact same genotype or are clones; and 3) locus dependency. In light of these difficulties in mouse ES cells, Wang and Zhou state that methods for gene targeting in somatic cells must be even more efficient (See page 3 of 8, right column, paragraph 2, in particular).

Wang and Zhou teach that although use of the positive-negative selection (PNS) vector method for gene targeting improves results in mouse ES cells, the improvement in somatic cells is a thousand-fold less. Unpredictability also lies in the use of the promoterless vector which should restrict expression only to those cells in which homologous recombination has occurred, however Wang and Zhou teach that

depending on whether the targeting vector integrated into the genome close to a strong promoter, cells which have undergone non-homologous recombination could be positively selected, thus generating false positives (see page 3 of 8, right column, last paragraph). There is unpredictability of success in using of the method of gene targeting for *in vivo* use or *ex vivo* use in nuclear transfer for creating of clones. The unpredictability lies in the ability of somatic cells to proliferate. Wang and Zhou disclose an art recognized issue of poor efficiency of use of cells which have been gene targeted by the described methods because of their senescence or poor potency for population expansion (see Page 4 of 8, left column, paragraphs 1 and 2, for example) and Norgren (page 3 of 8 paragraph 4, in particular). In addition, Norgren teaches that not all gene targeting events result in functional disruption of the gene of interest (See page 5 of 8, left column, paragraph 2, in particular).

Use of methods of disrupting genes of interest in somatic cells including somatic stem cells *in vivo* are also unpredictable due to art-recognized problems of inefficiency of gene targeting and low abundance of somatic stem cells (See Yanez and Porter, page 150, right column last paragraph, for example). Yanez and Porter teach that even use of PNS vectors and promoterless constructs may not be successful due to inability of weak markers to allow selection of a target gene with low expression levels (see page 153, right column, paragraph 2, for example).

2) State of the art. The art at the time the invention was made was poorly developed and art recognized issues remain even post-filing. Wang and Zhou disclose that it is recognized in the art that gene targeting in somatic cells is more difficult than in

stem cells. In addition, Wang and Zhou disclose that efficiency of gene targeting in somatic cells is still very low. As stated above, Wang and Zhou teach that some genes are impossible to target using promoterless targeting vectors due to the lack of active promoters for some genes in some cell types (see page 6 of 8, left column, conclusion paragraph, for example). Yanez and Porter state that *in vivo* gene therapy by gene targeting is not viable at present due to recognized problems in targeting frequency (see page 156, right column paragraph 2, for example).

3) Number of working examples. Applicants present two *in vitro* working examples in which Tumor necrosis factor receptor-associated factor (TRAF) was disrupted in B cell lines and in fibroblasts. Applicants have not presented any working examples of how the claimed vector and method might be used to disrupt a non-TRAF gene in cells *in vitro* or a gene of interest *in vivo* or in cells for use *ex vivo*.

4) Amount of guidance presented. Applicants have provided no specific guidance on how the method might be used to disrupt gene expression in a somatic cell *in vivo* such as for gene therapy or in a somatic cell for use in nuclear transfer cloning. Applicants have provided no guidance as to the number or type or size of gene which might be disrupted using the claimed method, or how many cells must show evidence that the gene has been disrupted in order to see an effect of the disruption of the gene of interest.

5) Scope of the invention. The invention is broad in that it involves a method for disrupting any gene of interest *in vitro, in vivo* or *ex vivo* in any somatic cell type.

6) Nature of the invention. The invention involves a very complex area of medicine/ molecular biology: targeted gene disruption for gene therapy or somatic cell cloning.

7) Level of skill in the art. The level of skill in the art is high: however, given the unpredictability of the art, poorly developed state of the art and lack of guidance presented by the applicants, the skilled artisan would have had to have practiced trial and error experimentation in order to practice the claimed invention.

Given the above analysis of the factors which the Courts have determined are critical in ascertaining whether a claimed invention is enabled, it must be considers that the skilled artisan would have had to have practiced undue and excessive experimentation in order to practice the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi et al (U.S. Patent No. 5,631,153, view of Sedivy et al. (Gene targeting and somatic cell genetics. 1999. Trends in Gen. 15(3):88-90). Applicants claims a somatic cell gene targeting vector comprising a gene targeting construct comprising a first

cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless; and an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence. The vector further comprises a first- and second- site specific recombination sequence such as loxP for a recombinase such as Cre recombinase where the recombination sequences flank the DNA encoding the positive selection marker The cloning sites comprise DNA segments that are homologous to genomic target sequences. Applicants claim a method for disrupting a gene of interest by introducing the claimed vector into a somatic cell so that the first and second genomic target sequences recombine with the gene to yield a genetically altered cell. Applicants claim isolated cells prepared by said methods and somatic cells comprising the vector.

Capecchi et al teach a vector to modify a target DNA sequence in the genome of a cell capable of homologous recombination comprising a first homologous vector DNA sequence capable of homologous recombination with a first region of a target DNA sequence, a promoterless positive selection marker sequence, a 2nd homologous vector DNA sequence capable of homologous recombination with a second region of the target DNA sequence, and a negative selection marker sequence (See abstract, column 5, lines 5-35, and column 9, lines 10-15, in particular). Capecchi et al disclose that expression of the negative selection marker can be under the control of a promoter known in the art (See column 13, lines 56-67 and Table IIB, for example) which reads on a vector with a weak promoter that is phosphoglycerate kinase (PGK). Capecchi et

al teach that the positive selection markers sequences can be neomycin (Neo) and that the negative selection marker can be diphtheria toxin (see column 7, lines 18-20, Table I, in particular) which reads on the claimed invention where the negative selection marker is diphtheria toxin. Capecchi et al teach that that said vector can be used in a method to inactivate genes by homologous integration of the positive selection sequence into the targeted gene and disrupting the expression of the gene in a cell which can be selected by way of the expression of the positive selection marker (Column 12, lines 34-52, column 13, lines 6-25 and Example 6, for example) which reads on disruption of a gene of interest in a cell by homologous recombination of the vector and the gene so that the genetically altered cell can be identified by expression of the positive selection markers. Capecchi et al also disclose first transformed target cells in which the vector has been homologously integrated into the target genome and also second transformed target cells produced by homologous recombination by which the positive selection marker is excised from the gene (see column 10, lines 49-66, bridging to column 11, lines 1-8, in particular). Capecchi et al exemplify the method in mouse embryonic stem cells, human bone marrow cells and plants, but disclose that said vectors and methods can be used with any cell type capable of homologous recombination including mammalian and human cells (see column 15, lines 41-55, for example). Capecchi et al do not teach that the site-specific recombination sequence is a Cre Lox system or the use of the vector in somatic cells. Capecchi et al do not teach that polyadenylation sequences flank the positive selection sequence.

Sedivy et al teach targeted homologous gene recombination in somatic cells

using positive negative selection (PNS) vectors and using promoterless vectors (see page 88, left column, paragraph 2, bridging to right column, and Figure 1 for example). Sedivy et al teach the use of a polyadenylation sequence with the PNS and promoterless vectors and also teach that the Cre-Lox system of recombination can be used for specific recombination (see Figure 1 and 2, and page 90, left column, paragraph 2). One of ordinary skill in the art would know that the term Lox refers to the recombination recognition sequence and the term Cre refers to Cre recombinase. It would have been obvious to one of ordinary skill in the art to modify the teaching of Capecchi et al of a vector and a method to target DNA sequences in somatic cells because Capecchi et al use the disclosed method in cells capable of homologous recombination and Sedivy et al teach that gene targeting is important in human somatic cells for use in gene knock-out experiments that might be ethically unacceptable in the human germ cell line. Sedivy et al also teaches that B cells are recombinogenic. It would also have been obvious to modify the teaching of Capecchi et al to include polyadenylation sequences and Lox recombinase recognition sites in the PNS vector because Capecchi et al teach that different regulatory sequences to modify gene expression can be used and combined (column 13, lines 65-67, in particular) and Sedivy et al teaches that polyadenylation sequences flank the positive and negative selection sequences in PNS vectors. Sedivy et al teaches the advantage of using the CreLox system of site-specific recombination especially when sequential targeting might be desired. The motivation to do so is the expected benefit as suggested by Capecchi et al and Sedivy et al of being able to target genes in somatic cells in order to

knock out genes in somatic cells. There is a reasonable expectation of success in using a PNS vector with a Cre-Lox recombination site in somatic cells since this has worked previously in cited techniques.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Laura McGillem, PhD
8/4/2005

DAVID GUZO
PRIMARY EXAMINER